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Subunit treadmilling of microtubules or actin in the presence of cellular barriers: Possible conversion of chemical free energy into mechanical work

(linear polymer/NTPase activity/chromosome movement/force–velocity curve/hemoglobin S)

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ABSTRACT **Free microtubule or actin filaments, along with the monomeric forms of the protein, hydrolyze GTP or ATP to produce a flux of subunits through the polymer. This flux, called treadmilling, produces no useful work. In the cell, however, these filaments are likely to be constrained between nucleating sites and other barriers that will limit polymer growth. We study here the effects of a small compression of the filaments resulting from polymerization against such barriers. If subunits can still exchange at the two ends, treadmilling will take place here as well. Under these conditions, the filament system can do useful work. The free energy of NTP hydrolysis can be used to transport materials, attached to the filament, against a resisting force. This process can in principle take place at high efficiency and bears a resemblance in a bioenergetic sense to the utilization of ATP free energy in muscle contraction. The same general principles apply to a polymer in which one end is anchored and one end is free.**

Steady-state treadmilling (1–3) of subunits in microtubules or actin microfilaments in solution uses GTP or ATP, respectively, but none of the NTP free energy loss is converted into mechanical work or other form of free energy (4). If the free subunit concentration is increased above the steady-state concentration, these polymers will grow (5). In a cell, a growing polymer, with one or both ends free, will eventually encounter a barrier of one kind or another (e.g., the cell membrane) at the free end or ends. At this point, further addition of a relatively small number of subunits will produce sufficient compression of the polymer to stop the net growth. This important effect is a consequence of the increase in the chemical potential of the subunits in the polymer; there is some resemblance here to the pressure-induced chemical potential increase of the solvent (in the solution) in an osmotic system. Although growth has ceased, a new regime of treadmilling will now be possible if subunits can still be exchanged between the solution and *both* ends of the polymer. In this paper we show that, if a chromosome or some other structure (or structures) is attached to the polymer, treadmilling between barriers, and exerts a force that resists the directional motion of the subunits of the polymer, then some of the NTP free energy of hydrolysis, expended in the treadmilling, will be converted into mechanical work. In fact, this latter system, in a formal bioenergetic sense, resembles the actin–myosin–ATP system in muscle contraction. For example, there will be a force–velocity curve: As the resisting force is increased, the treadmilling velocity decreases.

Treadmilling and free energy transfer of the above type is also possible if one end of the polymer is anchored and one end is free, although the details are different (to be published elsewhere).

Our object in this paper is to outline the basic bioenergetic theory applicable to a polymer that is treadmilling against barriers at both ends, with or without an attached resisting force. With the resisting force, work can be done. As a preliminary, however, we consider an equilibrium polymer (i.e., one with no NTPase activity) that has grown and encountered barriers, and consequently is under a compressive force. This equilibrium treatment would apply to other cases directly, for example, to sickle-cell hemoglobin (Hb S) aggregation.

EQUILIBRIUM POLYMER BETWEEN BARRIERS

We consider the aggregation of a linear polymer with no NTPase activity. Let c_e be the "critical" concentration of monomers (subunits) in equilibrium with an infinite polymer in solution. If the monomer concentration c is slightly $<c_e$, finite polymers are stable (4, 6, 7). If $c > c_e$, the polymers will grow steadily. We are interested here in the case in which $c > c_e$, but the polymer, in its growth, encounters barriers a distance L apart that limit its length. Monomer exchange is assumed to be possible at one or both ends despite the barriers. The polymer is somewhat compressible. The assumed compressibility, including bending, if any, is expressed by $F = a(l - l_0)$ (Hooke's law), where l_0 is L/N_0, l is L/N, F is the force on the polymer (negative for compression), a is a force constant, N is the number of monomers in the polymer, and N_0 is the value of N at zero force ($F = 0$). Hooke's law uses the first term in an expansion in powers of $l - l_0$; it is adequate for small compressions, as in the present problem.

The system (polymer) is characterized by the independent variables c, L, $a(T)$, $l_0(T)$, and the temperature T (N is a dependent variable that fluctuates). The independent thermodynamic variables are μ, L, and T, where μ is the chemical potential of monomers in the polymer (μ) or in the solution (μ_s):

$$\mu = \mu_s = \mu_s^0 + kT \ln c. \qquad [1]$$

For microtubules, $l_0 = 80/13 = 6.15$ Å; a is not known, but we make reasonable guesses below. Strictly, we should use the grand partition function $\Xi(\mu,L,T)$ and small system thermodynamics (6, 8) for this open finite system (with end effects included). However, N_0 is fairly large, of order 10^4 or 10^5, so with very small error we can use the canonical (or any) partition function and macroscopic thermodynamics.

The canonical partition function for the above defined system, omitting end effects, is

$$Q(N,L,T) = \left\{ q \exp\left[-a\left(\frac{L}{N} - l_0\right)^2 \Big/ 2kT \right] \right\}^N, \qquad [2]$$

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where $Q = q^N$ when the polymer is under no compression ($c = c_e$). The chemical potential in this case is

$$-kT \ln q \equiv \mu_0 = \mu_s^0 + kT \ln c_e, \quad [3]$$

so that

$$\mu - \mu_0 = kT \ln(c/c_e). \quad [4]$$

From the thermodynamic relation

$$dA = -SdT + FdL + \mu dN \quad [5]$$

and Eq. 2, we deduce

$$F = a(l - l_0) \quad [6]$$

$$\mu - \mu_0 = -\frac{a}{2}(l^2 - l_0^2) = -[l_0 F + (F^2/2a)]. \quad [7]$$

For a given value of $c/c_e > 1$, Eqs. 4 and 7 determine the equilibrium (compressive) force F. The corresponding value of N is also given by Eq. 7 because $l = L/N$. If the monomer concentration is increased from c_e to $c > c_e$, $-F$ and N increase from $-F = 0$ and $N = N_0$ until μ in the polymer becomes equal to $\mu_s(c)$ in the solution (Eq. 1).

The detailed balance relations for on–off transitions, at either end of the polymer, are $\alpha c = \alpha'$ for Eq. 1 and $\alpha_0 c_e = \alpha_0'$ for Eq. 3, where the αs are rate constants. These rate constants are related by (Eq. 4)

$$\alpha/\alpha' = (\alpha_0/\alpha_0')e^{(\mu_0 - \mu)/kT}. \quad [8]$$

The separate rate constants can be written as

$$\alpha = \alpha_0 e^{f(\mu_0 - \mu)kT}$$
$$\alpha' = \alpha_0' e^{(f-1)(\mu_0 - \mu)/kT}, \quad [9]$$

where f is an essential parameter (9) that represents the "split" of $\mu_0 - \mu$ between forward and backward transitions. Usually $0 \leq f \leq 1$, but this is not necessary.

The barriers referred to above need not be rigid. If they are somewhat elastic, they will be pushed back by the growing polymer until they resist with the same equilibrium force F (Eqs. 4 and 7). In this case, L refers to the polymer length when growth stops (equilibrium). A cap is a barrier that offers no mechanical resistance at all ($F = 0$), although rate constants will be affected. (This case will be discussed elsewhere.)

As already mentioned, N actually fluctuates, so that N in Eq. 7 is really the mean value $\bar{N}$. The variance in N, when L is held fixed, is (using Eq. 7)

$$\sigma_N^2 = \overline{N^2} - \bar{N}^2 = kT(\partial \bar{N}/\partial \mu)_{L,T} = kT\bar{N}^3/aL^2. \quad [10]$$

The Principal Approximation. There are approximate forms of the above relations, probably good to 1% or better (depending on a). We define n (number of subunit insertions) by $N = N_0 + n$. Then, if $\bar{n} \ll N_0$ (i.e., very little compression), which is presumably the case, the above equations simplify to

$$(\mu - \mu_0)/kT = \ln(c/c_e) = -l_0 F/kT = \gamma \bar{n} \quad [11]$$

$$\sigma_N^2 = \sigma_n^2 = 1/\gamma, \qquad \gamma \equiv al_0^2/N_0 kT, \quad [12]$$

where γ is a dimensionless force constant (Eq. 11) for subunit insertions (n) that is more convenient as a parameter than a. Because $\ln(c/c_e)$ is of order unity, the approximation is valid if $N_0 \gg \gamma^{-1}$. Eqs. 8, 9, and 11 are equivalent to equations 5–8 of ref. 9.

For a given value of c/c_e, both $\bar{n}$ and σ_n^2 vary as $1/\gamma$ or as $1/a$. For example, if $c/c_e = e = 2.72$ and $\gamma = 0.01$, than $\bar{n} = 100$ and $\sigma_n = 10$. If $\gamma = 0.05$, $\bar{n} = 20$ and $\sigma_n = 4.5$. Recall that N_0 is of order 10^4 or 10^5 and that $\bar{n} = 13$ would correspond to adding one subunit to each strand of a microtubule. The addition of 10–30 subunits for c/c_e of order 3–5 seems intuitively reasonable.

The estimation of the actual compressibility of a microtubule (or of a Hb S aggregate) is not easy. As an average for both torsional and center-of-mass vibrational motion in Hb S, using an Einstein model, Ferrone *et al.* (10) chose a frequency 3×10^9 s^{-1}. For the center-of-mass motion itself, we then estimate 8×10^9 s^{-1}. Then, by a straightforward argument to be published elsewhere, taking $N_0 = 2 \times 10^4$, we find $\gamma \cong 0.05$.

If we write Eq. 2 as $Q = q^N e^{-W/kT}$, then W is the work of inserting $\bar{n}$ subunits. W/kT is easily seen to be $\gamma \bar{n}^2/2$. Then the work per subunit added is

$$W/\bar{n} = \gamma \bar{n} kT/2 = (kT/2)\ln(c/c_e). \quad [13]$$

If $c/c_e = 2.72$, $W/\bar{n} = kT/2 \approx 300$ cal mol^{-1}. Note that $W/\bar{n}$ is independent of γ.

For reference below, we now consider this system from a more detailed stochastic point of view (in the same approximation). We are interested in the rate constants for the arbitrary polymerization step (at one end only) $n \rightleftarrows n + 1$. With no compression, these rate constants are $\alpha_0 c$ and α_0' (Eq. 8). The work of insertion for $n \rightarrow n + 1$ is

$$\Delta W/kT = (\gamma/2)[(n+1)^2 - n^2] = (2n+1)\gamma/2. \quad [14]$$

On including this effect, the two rate constants become (Eq. 9)

$$\alpha_0 c x^{(2n+1)f} \text{ and } \alpha_0' x^{(2n+1)(f-1)}, \quad [15]$$

where $x \equiv e^{-\gamma/2}$. If P_n is the equilibrium probability of a polymer with n subunit insertions, then detailed balance in $n \rightleftarrows n + 1$ together with Eq. 15 gives

$$P_{n+1}/P_n = \alpha_0 c x^{2n+1}/\alpha_0'. \quad [16]$$

On the other hand, from the grand partition function for an open system with N molecules, $P_N \approx Q(N)\lambda^N$, where $\lambda = e^{\mu/kT}$. Thus, in the present case (Eq. 2),

$$P_n \sim (q\lambda)^n x^{n^2}, \qquad P_{n+1}/P_n = q\lambda x^{2n+1}. \quad [17]$$

On comparing this with Eqs. 4 and 16, we verify that

$$q\lambda = c/c_e = \alpha_0 c/\alpha_0', \quad [18]$$

which is a self-consistency check.

The equilibrium distribution P_n will be substantially confined to positive n if $\bar{n} > 3\sigma_n$—that is, if

$$\ln(c/c_e) > 3\gamma^{1/2}. \quad [19]$$

This will usually be the case.

The parameter f in Eqs. 9 and 15 could in principle be a function of n (or of F), but it is a reasonable approximation to treat it as a constant for $n > 0$ (9). This is done below; here, at equilibrium, the choice is immaterial.

TREADMILLING BETWEEN BARRIERS

We now consider a polymer with on–off NTPase activity at each end (1, 4) but without a resisting force ($F_r = 0$ in Fig. 1). The monomer or subunit concentration c is large enough ($c > c_\infty$; see below) so that the polymer grows until it is restrained by barriers, as discussed above, and as shown schematically in Fig. 1. This is presumably a common situation *in vivo* for both tu-

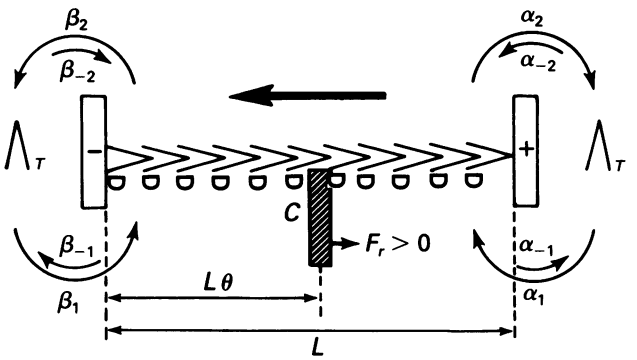

FIG. 1. Schematic representation of a steady-state treadmilling polymer between two barriers a distance L apart. Λ is a monomer, Λ_T (in solution) contains bound NTP, and Δ_D (in polymer) has bound NDP. The end monomers of the polymer can exchange with Λ_T in solution. The cycle biochemistry is discussed in refs. 4 and 11. There is net addition of monomer at the + end and net loss at the − end. The heavy arrow shows direction of motion of monomer in polymer; ▨, appendage C, which resists this motion with a force F_r.

bulin and actin. The polymer has length L and is under a compressive force F just sufficient to halt the net growth, but treadmilling of subunits is assumed to occur. Under these conditions, the rate constant notation is as shown in Fig. 1. Equations 6–17 of ref. 4 apply without formal change except that $\bar{c}_1$ is replaced here by c, e.g.,

$$c = (\alpha_2 + \beta_2 + \alpha_{-1} + \beta_{-1})/(\alpha_1 + \beta_1 + \alpha_{-2} + \beta_{-2}) \quad [20]$$

is the condition for zero net growth of the polymer. When $F = 0$ ($c = c_\infty$ below), the rate constants are denoted by α_1^0, β_1^0, etc. These would differ from the rate constants for a polymer with free ends because of the "capping." Equations 6–17 of ref. 4 also apply with the $F = 0$ set α_1^0, β_1^0, etc; in this case, we denote the steady-state monomer concentration by c_∞:

$$c_\infty = (\alpha_2^0 + \beta_2^0 + \alpha_{-1}^0 + \beta_{-1}^0)/(\alpha_1^0 + \beta_1^0 + \alpha_{-2}^0 + \beta_{-2}^0). \quad [21]$$

In principle, each transition pair in Fig. 1 (e.g., α_1, α_{-1}) can be considered in an equilibrium with polymer by itself, as in Eqs. 6–9. Thus, we can write the explicit rate constant equations (see equations 22 and 23 of ref. 9)

$$\begin{aligned} \alpha_1 &= \alpha_1^0 \varepsilon^{f_1^\alpha}, & \alpha_{-1} &= \alpha_{-1}^0 \varepsilon^{f_1^\alpha - 1}, \\ \alpha_2 &= \alpha_2^0 \varepsilon^{-f_2^\alpha}, & \alpha_{-2} &= \alpha_{-2}^0 \varepsilon^{1-f_2^\alpha}, \\ \beta_1 &= \beta_1^0 \varepsilon^{f_1^\beta}, & \beta_{-1} &= \beta_{-1}^0 \varepsilon^{f_1^\beta - 1}, \\ \beta_2 &= \beta_2^0 \varepsilon^{-f_2^\beta}, & \beta_{-2} &= \beta_{-2}^0 \varepsilon^{1-f_2^\beta}, \end{aligned} \quad [22]$$

where four different fs are introduced for generality and $\varepsilon = e^{l_0 F/kT}$ in the approximation above ($\bar{n} \ll N_0$) that we shall continue to use (Eq. 7 gives the correction term in the definition of ε if the approximation is not used). In principle, the fs can all be functions (9) of F, but we shall treat them as constants below.

In a general numerical calculation, $c > c_\infty$ is first specified. Eqs. 22 are then substituted into Eq. 20 and that value of $F < 0$ is found which satisfies Eq. 20. At the same time, using this F, values of all rate constants in Eqs. 22 are determined. These are then introduced into equations 4–17 of ref. 4 to calculate the monomer flux J_m, the NTP flux J_T, etc. Also, using F, the value of $\bar{n}$ can be found from Eq. 6, because $l = L/(N_0 + \bar{n})$. Eq. 6 is a mechanical property of the polymer that holds whether the polymer is at equilibrium or at steady state.

We have the usual thermokinetic relations (4)

$$\begin{aligned} \alpha_1^0\alpha_2^0/\alpha_{-1}^0\alpha_{-2}^0 = \beta_1^0\beta_2^0/\beta_{-1}^0\beta_{-2}^0 &= e^{X_T/kT} \\ X_T &= \mu_T - \mu_D - \mu_P, \end{aligned} \quad [23]$$

where X_T is the NTP thermodynamic force (denoted by X in ref. 4). Eqs. 23 also hold for the rate constants in Eqs. 22 ($F \neq 0$).

Special Case. We turn now to the most important special case. In addition to assuming that $\bar{n} \ll N_0$, we drop rate constants with negative subscripts and take $f_1^\alpha = f_1^\beta \equiv f_1$ and $f_2^\alpha = f_2^\beta \equiv f_2$ [this is likely because the chemical steps in the NTPase activity (4, 11) are probably the same at the two ends of the polymer]. Eqs. 20–22 then lead to

$$(f_1 + f_2)^{-1}\ln(c/c_\infty) = -l_0F/kT = \gamma\bar{n} \quad [24]$$

for F and $\bar{n}$ as functions of c/c_∞. The NTP and monomer fluxes are (4)

$$J_T = \alpha_2 + \beta_2 = J_T^0(c/c_\infty)^{f_2/(f_1+f_2)} \quad [25]$$

$$J_m = (\alpha_1\beta_2 - \alpha_2\beta_1)/(\alpha_1 + \beta_1) = J_m^0(c/c_\infty)^{f_2/(f_1+f_2)}, \quad [26]$$

where $J_T^0 = \alpha_2^0 + \beta_2^0$ and

$$J_m^0 = (\alpha_1^0\beta_2^0 - \alpha_2^0\beta_1^0)/(\alpha_1^0 + \beta_1^0). \quad [27]$$

Thus J_T and J_m both increase with c/c_∞ (assuming f_1 and f_2 are positive, as would be expected) by the same factor. As a first guess, one might take $f_1 = f_2 = 1/2$.

Stochastic Treatment in the Special Case. Because the kinetic scheme is linear (in n), a "detailed balance" type of solution (11) is possible (numerically) at steady state, even in the most general case. But in the special case above (Eqs. 24–27), we can give an analytical quasi-equilibrium treatment. If we use Eq. 15 for each transition pair together with the f notation in Eqs. 22, the rate constants for $n \rightleftarrows n + 1$ become (in place of Eq. 15)

$$c(\alpha_1^0 + \beta_1^0)x^{(2n+1)f_1} \quad \text{and} \quad (\alpha_2^0 + \beta_2^0)x^{-(2n+1)f_2}. \quad [28]$$

Thus, at steady state,

$$P_{n+1}/P_n = (c/c_\infty)y^{2n+1}, \quad [29]$$

where $y \equiv x^{f_1+f_2}$. This is formally the same as Eq. 16 or 17 with y in place of x and c/c_∞ in place of $\alpha_0 c/\alpha_0'$ or $q\lambda$. Hence, $P_n \sim (c/c_\infty)^n y^{n^2}$. This P_n, put in gaussian form, then gives the same $\bar{n}$ as a function of c/c_∞ as in Eq. 24 and, in addition, $\sigma_n^2 = 1/\gamma(f_1 + f_2)$. A stochastic approach is necessary to obtain this steady-state result. Also, we can make a correction to J_T and J_m (Eqs. 25 and 26) to take care of the fact that this is actually an open system with fluctuations in n. We find, after a short calculation,

$$\begin{aligned} J_T &= c(\alpha_1^0 + \beta_1^0)\sum_n P_n x^{(2n+1)f_1} \\ &= J_T^0(c/c_\infty)^{f_2/(f_1+f_2)} e^{-\gamma f_1 f_2/2(f_1+f_2)}. \end{aligned} \quad [30]$$

A similar calculation of J_m gives the same correction factor in γ. The correction is probably of order 1% or less. The treatment in Eqs. 24–27 corresponds to using only the term $n = \bar{n}$ in the sums over n encountered in this subsection.

TREADMILLING BETWEEN BARRIERS AGAINST A RESISTING FORCE

Here we consider the more general and interesting bioenergetics of the treadmilling polymer in Fig. 1 on which there is

an appendage C that moves with the subunits against a resisting force F_r. There has been much interest in possible ways that treadmilling NTPase activity might be put to some use (5). The scheme in Fig. 1, with F_r included, allows the transfer of some (or all, in a limiting case) NTPase free energy of hydrolysis into the mechanical work of moving C against F_r. For example, C might be a vesicle or chromosome that is transported by a microtubule and F_r its viscous drag [although F_r in this case would be very small, $\approx 10^{-8}$ dyne (1 dyne = 0.01 mN) for a particle the size of a chromosome (12, 13)]. The position of C, specified by the fraction θ in Fig. 1, does not appear in the final results (see below). C could also represent a composite of a number of attachments.

Our primary interest here is not in particular applications but in general principles: we shall consider that F_r can be made arbitrarily large, even large enough to stop or to reverse the treadmilling direction.

If one subunit is inserted at the + end of the polymer in Fig. 1, C moves to the left essentially a distance θl_0 against the force F_r. From this and similar considerations for other possible cases, we must now generalize Eqs. **22** to read

$$\begin{aligned} \alpha_1 &= \alpha_1^0 \varepsilon^{f_1^\alpha} r^{-\theta f_1^\alpha}, & \alpha_{-1} &= \alpha_{-1}^0 \varepsilon^{f_1^\alpha - 1} r^{\theta(1-f_1^\alpha)} \\ \alpha_2 &= \alpha_2^0 \varepsilon^{-f_2^\alpha} r^{\theta f_2^\alpha}, & \alpha_{-2} &= \alpha_{-2}^0 \varepsilon^{1-f_2^\alpha} r^{\theta(f_2^\alpha - 1)} \\ \beta_1 &= \beta_1^0 \varepsilon^{f_1^\beta} r^{(1-\theta)f_1^\beta}, & \beta_{-1} &= \beta_{-1}^0 \varepsilon^{f_1^\beta - 1} r^{(1-\theta)(f_1^\beta - 1)} \\ \beta_2 &= \beta_2^0 \varepsilon^{-f_2^\beta} r^{-(1-\theta)f_2^\beta}, & \beta_{-2} &= \beta_{-2}^0 \varepsilon^{1-f_2^\beta} r^{(1-\theta)(1-f_2^\beta)}, \end{aligned} \quad [31]$$

where $r \equiv e^{l_0 F_r/kT}$. Here $r \geq 1$ and $\varepsilon \leq 1$. The value of r is assigned in advance, along with c. ε and θ occur in Eqs. **31** only in the combination $\varepsilon r^{-\theta}$. The value of $\varepsilon r^{-\theta}$ is that which satisfies the steady-state Eq. **20** when Eqs. **31** are substituted there. If this value of $\varepsilon r^{-\theta}$ is used, Eqs. **31** at steady state no longer depend explicitly on ε and θ.

The six steady-state cycles (14) that have to be considered are indicated in Fig. 2, along with the thermodynamic force in each case. The relations between these forces and rate constants are (using Eqs. **23**):

$$\begin{aligned} &\text{(A) } \alpha_1\alpha_2/\alpha_{-1}\alpha_{-2} = e^{X_T/kT}, && \text{(B) } \beta_1\beta_2/\beta_{-1}\beta_{-2} = e^{X_T/kT} \\ &\text{(C) } \alpha_1\beta_2/\alpha_{-1}\beta_{-2} = e^{X_T/kT} r^{-1}, && \text{(D) } \alpha_2\beta_1/\alpha_{-2}\beta_{-1} = e^{X_T/kT} r \\ &\text{(E) } \alpha_1\beta_{-1}/\alpha_{-1}\beta_1 = r^{-1}, && \text{(F) } \alpha_{-2}\beta_2/\alpha_2\beta_{-2} = r^{-1}. \end{aligned} \quad [32]$$

Cycles A and B hydrolyze NTP, cycles C and D hydrolyze NTP and move C, whereas cycles E and F only move C.

The fluxes can be found from (4)

$$\begin{aligned} J_T &= (\alpha_1 c - \alpha_{-1}) + (\beta_1 c - \beta_{-1}) \\ J_m &= (\alpha_1 + \alpha_{-2})c - (\alpha_{-1} + \alpha_2). \end{aligned} \quad [33]$$

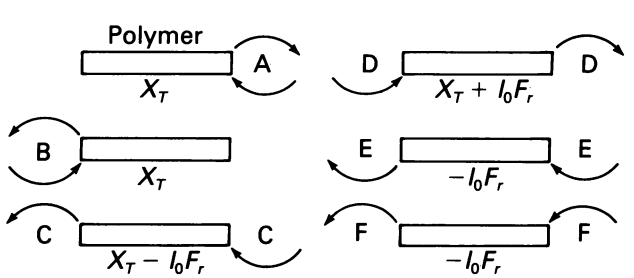

FIG. 2. The fluxes J_T and J_m in Eqs. **34** can be decomposed into the six cycle fluxes given in Eqs. **35**. These cycle fluxes are indicated graphically (compare rate constants in Eqs. **35** with those in Fig. 1). A rectangle represents the polymer of Fig. 1. Under each rectangle is the thermodynamic force associated with the indicated cycle (Eqs. **32**).

On substituting Eq. **20** and using Eqs. **32**, we find

$$J_T = J_A + J_B + J_C + J_D, \qquad J_m = J_C - J_D + J_E + J_F, \quad [34]$$

where the subscripts identify the cycle (Fig. 2) and

$$\begin{aligned} &J_A = \alpha_1\alpha_2(1 - e^{-X_T/kT})/D, \quad J_B = \beta_1\beta_2(1 - e^{-X_T/kT})/D \\ &J_C = \alpha_1\beta_2(1 - e^{-X_T/kT} r)/D, \quad J_D = \alpha_2\beta_1(1 - e^{-X_T/kT} r^{-1})/D \\ &J_E = \alpha_1\beta_{-1}(1 - r)/D, \qquad J_F = \alpha_{-2}\beta_2(1 - r)/D \\ &\qquad D \equiv \alpha_1 + \beta_1 + \alpha_{-2} + \beta_{-2}. \end{aligned} \quad [35]$$

The terms in $e^{-X_T/kT}$ are generally negligible as are the rate constants that have negative subscripts.

The efficiency of free energy transduction and the rate of free energy dissipation are

$$\eta = J_m l_0 F_r / J_T X_T \quad [36]$$

$$T(d_i S/dt) = J_T X_T - J_m l_0 F_r \quad [37]$$

$$= (J_A + J_B)X_T + J_C(X_T - l_0F_r) + J_D(X_T + l_0F_r) + (J_E + J_F)(-l_0F_r). \quad [38]$$

The latter expression is in terms of cycle fluxes and forces. Eqs. **36** and **37** have the same form as in the theory of muscle contraction (14), as might be expected, with $J_m l_0$ equivalent to the velocity of contraction and F_r equivalent to the load being lifted. $T(d_i S/dt)$ can also be expressed in terms of the four transition fluxes (Fig. 1) and gross free energy levels (4, 14), but we omit this.

Incidentally, it is possible in principle to have treadmilling in a polymer without NTPase activity. For example, if we put α_2, α_{-2}, β_2, and β_{-2} equal to zero above, cycle E still remains. The force (pulling, now, rather than resisting) in this cycle (Fig. 2) is $-l_0F_r$ and $J_m = J_E$ (negative): the force F_r induces treadmilling in the reverse direction from that in Fig. 1. There is no free energy transduction.

Tight Coupling. Because $r \geq 1$, the only positive term in J_m is J_C. In J_T, only the flux J_C moves C *against* F_r and hence contributes to free energy transduction. Thus, all cycles are completely wasteful (reduce η) except cycle C. Consider now the *hypothetical* case in which β_1^0, β_{-1}^0, α_2^0, and α_{-2}^0 are all zero (see Fig. 1). Thus only cycle C remains. There is tight coupling between NTPase activity and the movement of C: $J_T = J_m = J_C$. The efficiency becomes $\eta = l_0F_r/X_T$. If F_r is increased until l_0F_r approaches X_T, $\eta \to 1$. In this limit, the free energy transduction from NTP to C is complete. When $l_0F_r = X_T$, the system is at equilibrium: both the cycle C thermodynamic force, $X_T - l_0F_r$, and the flux, J_C, are zero. If $l_0F_r > X_T$, the treadmilling direction reverses and NTP is synthesized ($J_C < 0$).

Special Case. We consider again the important special case introduced above, but we now include F_r. Cycles E and F drop out; cycles A–D all become one-way cycles. From Eqs. **20** and **31**, the steady-state F and $\bar{n}$ are given by

$$(f_1 + f_2)^{-1} \ln(c/c') = -l_0F/kT = \gamma\bar{n} \quad [39]$$

where

$$c' = (\alpha_2^0 + \beta_2^0 r^{-f_2}) r^{\theta(f_1+f_2)} / (\alpha_1^0 + \beta_1^0 r^{f_1}). \quad [40]$$

Note that, for given c and r, F and $\bar{n}$ depend on θ (i.e., on the location of C along the polymer); in fact, $-F$ = constant $- \theta F_r$.

Using this F (Eq. 39), we then find for the steady-state fluxes

$$J_T = (\alpha_2^0 + \beta_2^0 r^{-f_2})\,[c(\alpha_1^0 + \beta_1^0 r^{f_1})/(\alpha_2^0 + \beta_2^0 r^{-f_2})]^{f_2/(f_1+f_2)} \quad [41]$$

$$J_m = (\alpha_1^0\beta_2^0 r^{-f_2} - \alpha_2^0\beta_1^0 r^{f_1})\,[c(\alpha_1^0 + \beta_1^0 r^{f_1})/(\alpha_2^0 + \beta_2^0 r^{-f_2})]^{f_2/(f_1+f_2)}/(\alpha_1^0 + \beta_1^0 r^{f_1}). \quad [42]$$

J_T and J_m do not depend on θ (see the discussion of Eqs. 31). J_m is zero when

$$r = r_0 \equiv (\alpha_1^0\beta_2^0/\alpha_2^0\beta_1^0)^{1/(f_1+f_2)}. \quad [43]$$

A plot of ln r against J_m is essentially the "force–velocity curve" (14) for this system.

In the tight-coupling special case ($\beta_1^0 = \alpha_2^0 = 0$),

$$J_T = J_m = \beta_2^0(c/c_\infty)^{f_2/(f_1+f_2)} r^{-f_1 f_2/(f_1+f_2)}, \quad [44]$$

where $c_\infty = \beta_2^0/\alpha_1^0$.

We conclude with a numerical example that illustrates Eqs. 39–43. We take, for a microtubule, $f_1 = f_2 = 1/2$, $c = 5.25\ \mu$M, and

$$\alpha_1^0 = 7\ \mu\text{M}^{-1}\cdot\text{s}^{-1}, \quad \alpha_2^0 = 7\ \text{s}^{-1}, \qquad \beta_1^0 = 1\ \mu\text{M}^{-1}\cdot\text{s}^{-1}, \quad \beta_2^0 = 7\ \text{s}^{-1}. \quad [45]$$

Then,

$$c_\infty = 1.75\ \mu\text{M}, \quad J_T^0 = 14\ \text{s}^{-1}, \qquad J_m^0 = 5.25\ \text{s}^{-1}, \quad c/c_\infty = 3, \quad r_0 = 7. \quad [46]$$

For the fluxes at c,

$$r = 1, \quad F_r = 0: J_T = 24.2\ \text{s}^{-1}, \quad J_m = 9.1\ \text{s}^{-1}, \qquad r = r_0 = 7: J_T = 22.1\ \text{s}^{-1}, \quad J_m = 0. \quad [47]$$

At $r = 1$, both fluxes are increased over the c_∞ values (Eq. 46) by a factor of $\sqrt{3}$ (Eqs. 25 and 26). A plot (not shown) of ln $r = l_0F_r/kT$ against J_m (Eq. 42) between the two points in Eq. 47 is *very* close to linear [unlike the force–velocity curve in muscle (14)]. The value of F_r ("isometric force," Eq. 43) at which $J_m = 0$ is 1.3×10^{-6} dyne (25°C), compared with $\approx 10^{-8}$ dyne for the drag of a chromosome (12, 13). The maximum treadmilling velocity (i.e., at $F_r = 0$) in this example is $9.1 \times 6.15 = 56$ Å·s^{-1} compared with $\approx$20 Å·ms^{-1} for V_{max} in muscle contraction and $\approx$170 Å·s^{-1} (or 1 μm·min^{-1}) for a chromosome in anaphase. The efficiency η (Eq. 36) is zero at $F_r = 0$ and at $J_m = 0$. In between, η has a maximum of only 0.81% (using $X_T/kT = 23.0$) at $r = 2.67$: most of the GTP hydrolysis is wasted. At this r, $J_m/J_T = 0.19$ and $l_0F_r/X_T = 0.043$. The low efficiency is implicit in this example at the outset because of the use of one-way (irreversible) cycles and the contributions of the inefficient cycles A, B, and D. The corresponding efficiency in muscle contraction is of order 50% (15).

At $r = 2.67$, $c' = 2.14\ \mu$M for $\theta = 1/2$ (Eq. 40). Then $-l_0F/kT = 0.90$ (Eq. 39). Also, $\bar{n} = 18.0$ if we take $\gamma = 0.05$ (Eq. 39).

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